

# Rapid Start-up and Loading of an Attached Growth, Simultaneous Nitrification/Denitrification Membrane Aerated Bioreactor

Caitlin E. Meyer<sup>1</sup>, Stuart Pensinger,<sup>2</sup> Karen D. Pickering<sup>3</sup>  
Daniel Barta<sup>4</sup>, and Sarah A. Shull<sup>5</sup>  
*Johnson Space Center, Houston, TX, 77058*

Leticia M. Vega<sup>6</sup>  
*Jacobs Engineering, Houston, TX, 77058*

Dylan Christenson<sup>7</sup> and W. Andrew Jackson<sup>8</sup>  
*Texas Tech University, Lubbock, TX, 79407*

**Membrane aerated bioreactors (MABR) are attached-growth biological systems used for simultaneous nitrification and denitrification to reclaim water from waste.** This design is an innovative approach to common terrestrial wastewater treatments for nitrogen and carbon removal and implementing a biologically-based water treatment system for long-duration human exploration is an attractive, low energy alternative to physiochemical processes. Two obstacles to implementing such a system are (1) the “start-up” duration from inoculation to steady-state operations and (2) the amount of surface area needed for the biological activity to occur. The Advanced Water Recovery Systems (AWRS) team at JSC explored these two issues through two tests; a rapid inoculation study and a wastewater loading study. Results from these tests demonstrate that the duration from inoculation to steady state can be reduced to under two weeks, and that despite low ammonium removal rates, the MABRs are oversized.

## Nomenclature

<i>AWP</i>	= Alternative Water Processor
<i>AWRS</i>	= Advanced Water Recovery Systems
<i>BWP</i>	= Biological Water Processor
<i>DI</i>	= Deionized
<i>IC</i>	= Ion Chromatography
<i>ISS</i>	= International Space Station
<i>JSC</i>	= Johnson Space Center
<i>KSC</i>	= Kennedy Space Center
<i>LEO</i>	= Low Earth Orbit

<sup>1</sup> Advanced ECLSS Technology Development Group, EC3/Crew and Thermal Systems Division, NASA Johnson Space Center

<sup>2</sup> Advanced ECLSS Technology Development Group, EC3/Crew and Thermal Systems Division, NASA Johnson Space Center

<sup>3</sup> Deputy Chief, Thermal Systems Branch, EC6/Crew and Thermal Systems Division, NASA Johnson Space Center

<sup>4</sup> Project Manager, Next Generation Life Support, EC1/Crew and Thermal Systems Division, NASA Johnson Space Center

<sup>5</sup> Lead Advanced ECLSS Technology Development Group, EC3/Crew and Thermal Systems Division, NASA Johnson Space Center

<sup>6</sup> Scientist, JETS Contract, 2224 Bay Area Blvd., Houston TX 77058.

<sup>7</sup> Research Assistant, Civil, Environmental and Construction Engineering, MS 41023

<sup>8</sup> Professor and Associate Chair, Civil and Environmental Engineering, MS 41023

<i>MABR</i>	= Membrane Aerated Bioreactor
P/C	= Physiochemical
<i>SOA</i>	= State of the Art,
TIC	= Total Inorganic Carbon
TN	= Total Nitrogen
TOC	= Total Organic Carbon
TTU	= Texas Tech University
VCD	= Vapor Compression Distillation

## I. Introduction

WATER is the most precious resource to enable human exploration of space beyond low earth orbit (LEO) and in order to provide the necessary water for a given mission, water recovery from waste is paramount. There are two general mechanisms that are used to reclaim water from wastewater. The first are strictly physiochemical (P/C) processes. The current state of the art (SOA) P/C process is vapor compression distillation (VCD), which is currently used on the International Space Station (ISS) to reclaim water from urine and humidity condensate. Physiochemical processes, such as distillation, typically require elevated temperatures and occur at non-atmospheric pressures; that is the process occurs under elevated pressure or vacuum.

An alternative to a strictly P/C system is a biologically-based process which uses bacteria to transform the wastewater constituents into products which can be reused for other life support activities. Biological systems require less energy than P/C systems; they use natural processes to break down the wastewater, operate at ambient temperatures, and require only slightly elevated pressures for microgravity compatibility. Because the constituents are transformed rather than concentrated, the toxic chemicals that are used to stabilize the wastewater are not needed.

While biological systems provide many advantages as primary processors, there are a number of hurdles to implementation. One is the time needed to produce a stable community of organisms capable of breaking down the wastewater. The second, maximizing the surface area needed within the reactor for that process to occur to reduce the volumetric footprint of the system. As a part of the Alternative Water Processor (AWP) testing, the test team evaluated these two issues.

## II. Materials and Methods

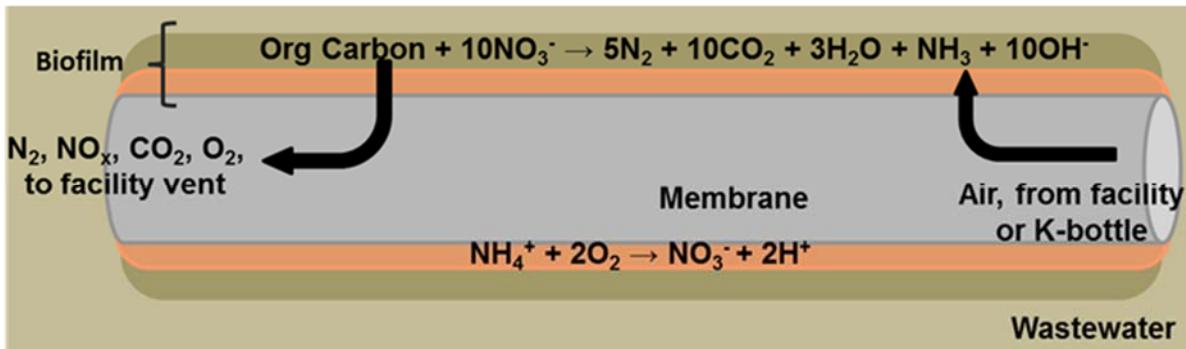
### A. Membrane Aerated Bioreactor (MABR)

The biological system was an attached growth membrane design known as a membrane aerated bioreactor (MABR). Each MABR was made up of two overlapping shells. The oxygen module contained 506 Silastic® tubes that ran the length of the module. Oxygen headers at the top and bottom of each reactor connected the reactor to the inlet air supply and product gas line. Two acrylic panels, or “clamshells,” provided the structural support caused by the tension induced by the Silastic® tubes. The clamshell was then placed inside a second acrylic cylinder to provide a pressure tolerant housing for the fluid (Figure 1).<sup>1</sup>

The Silastic® membranes provided surface area for the bacteria to attach to and for the subsequent biological reactions to take place. Air would flow from the oxygen header through the membranes. As the air traveled to the headers, oxygen would permeate through the membranes. Aerobic nitrifying bacteria would then form a biofilm on the surface of each of these membranes. As the biofilm thickened, the organisms in the biofilm farther from the membrane and the bacteria in the bulk fluid were unable to access the oxygen and relied on nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) as electron acceptors for carbon oxidation. The product gasses from denitrification, carbon dioxide ( $\text{CO}_2$ ) and nitrogen ( $\text{N}_2$ ) gas, were then scrubbed from the reactors, through the membranes, and out the effluent gas header to vent. An illustration of this process is given in Figure 2.



**Figure 1. Assembled and installed MABR**



**Figure 2. Biological reactions occurring at the surface of the membrane and in the biofilm**

### B. Inoculum

The inoculum was from a tank generated from a seed culture donated from the Jackson lab at TTU. The consortium within the tank were fed a dilute urine (10% vol) solution to acclimate the organisms to the nitrogen load. Once the consortium was acclimated, the volume increased by adding the same dilute strength until there was sufficient volume to fill both reactors with at least a 25% (by vol) margin. At the beginning of the rapid start evaluation, 200 liters of inoculum was available for use.

### C. Wastewater

The wastewater for the rapid start evaluation was initially a diluted urine solution. As in the inoculation tank in the above section, the rationale was to condition the organisms to a high nitrogen content to establish a nitrifying community within the reactors to convert the ammonium, created as a result of urea hydrolysis, to nitrite and nitrate for carbon oxidation.

**Table 1 describes the acclimation of the microorganisms in the MABRs from dilute urine to a full wastewater stream,**

Table 2 is the composition of the combined wastewater solution on a per person, per day basis. Once the reactor was able to successfully handle the one person wastewater load, the test team increased the volume processed per day until the effluent pH rose above 7.8, the point at which free ammonia began to inhibit nitrification at the concentration measured in the effluent samples.<sup>2</sup>

**Table 1. Stepwise feeding of the MABR**

Step	Volume fraction (% added of daily feed volume, 14.5L)	Influent composition	Volume	Feed Rate
First	50%	Urine in DI	7.5 L total (1.14L urine)	5.0 ml/min
Second	75%	Urine in DI	10.9L total: (1.7L urine)	7.6 ml/min
Third	100%	Urine in DI	14.5L total: (2.275L urine)	10.1 ml/min
Fourth	100%	Full wastewater combined stream	14.5L full combined wastewater	10.1 ml/min

**Table 2. Wastewater composition, per person, per day**

<b>Wastewater (WW) Type</b>	<b>WW Per day (liters)</b>	<b>Personal Care Products</b>
Urine	2.275	
<i>Hygiene</i>	Oral Hygiene	Arm & Hammer Toothpaste
	Hand Wash	No-Rinse Shampoo, NASA Formulation
	Shower	
	Shave	Neutrogena Men Shave Cream
	Urinal Flush	
Humidity Condensate	0.875	
Laundry	3.75	Seventh Generation Natural 2X Concentrated Laundry Liquid (Free and Clear)
<b>Total</b>	14.4375 (rounded to 14.5)	

**D. Analysis**

Samples from the feed tank and GLS were collected daily for pH, TOC/TIC, TN, and IC. In addition, a bioluminescent dissolved oxygen (Hach, 9020000) and pH sensors (Hach, DPD1P1) were installed in the recycle loop of each MABR system to provide real-time data on the operation of the system. Test points from the inline probes were collected each second during testing.

**III. Rapid Start Evaluation**

Establishing a robust nitrifying biofilm on the surface of the membranes is the key component with getting a MABR fully operational. Nitrifying bacteria are autotrophic slow growing organisms and as such, are the linchpin for reducing overall inoculation time. During the AWP test in 2013, it took 71 days for the BWP to go from inoculation to be able to process a complete wastewater. For human exploration applications, that duration is unacceptable. The objective of the Rapid Start-Up Test was to identify methods to reduce that duration. The test had two components: validate an inoculation method developed by TTU and determine an optimum membrane surface treatment to facilitate bacterial attachment and subsequent biofilm development.

**A. Method Validation**

Researchers at Texas Tech University (TTU) developed a method to inoculate a MABR that greatly reduced the duration between inoculation and the processing of a full waste stream. A MABR was filled with a filtered (100µm) inoculum solution acclimated to urine. The system was operated in recycle until the pH dropped below 6. After two additional successful smaller inoculations, 25% and 20% of the volume respectively, the MABR was gradually fed higher concentrations of urine corresponding to higher nitrogen concentrations, as long as the pH was in a range of 6.5-7.8. Once the system could process a day's volume of urine in a day, the hygiene wastewater was added and the system was considered fully operational. The method was successful at reducing the time between inoculation and nominal operations: 23 days, as compared to 71 days for the AWP test<sup>2</sup>. The goal at JSC was to replicate or improve on those results.

**B. Membrane Modification: Chemical Etching**

A second way to reduce the time from the inoculation to steady state operations is to enhance attachment of bacteria onto the surface of the membrane. As part of the FY13 Alternative Water Processor testing, Kennedy Space Center performed an assessment of various surface modifications of the Silastic™ membranes. The goal was to identify either a mechanical or chemical treatment method that would promote biofilm adhesion. They determined that submerging the membranes in a specialty solvent for several seconds ("Fluoroetch 18 second method") modified the membranes such that it allowed bacteria to more easily adhere to the surface without affecting its mechanical or

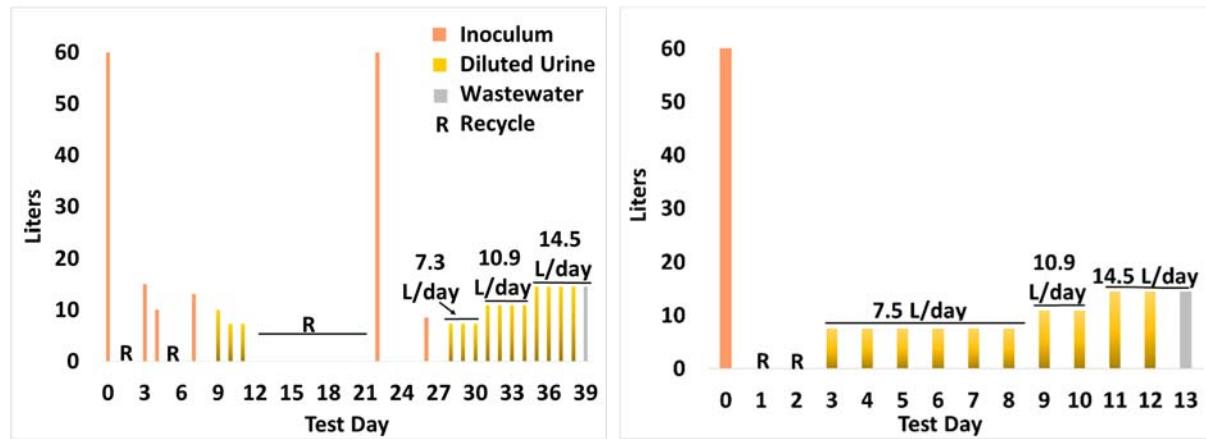
gas transport properties. Using inoculum obtained from TTU, KSC demonstrated that this method resulted in the highest concentration of attached cells among the tested membranes<sup>3</sup>.

### C. Membrane Modification: Conditioned Tubing

The second surface modification treatment that was evaluated was the use of conditioned membranes. A second MABR was drained and the membranes rinsed with deionized (DI) water to remove the loosely attached biomass from the surface. It was anticipated that the biofilm previously present, as well as the bacteria remaining on the surface of the membranes, conditioned the surface of the membrane to make re-colonization easier and subsequently decrease the time for the nitrifying bacteria to establish a biofilm.

### D. Testing and Results

Testing began on July 9<sup>th</sup>, 2014. Two reactors, one containing etched membranes and one containing conditioned membranes were filled with 60 liters of filtered inoculum, which was fed a dilute urine solution just prior to filling. The reactors were pressurized to 14 psi(g) air and 15psi(g) liquid pressure and operated on internal recycle until there was a sufficient drop in the pH of the recycle loop, indicating nitrification had begun and stepwise feeding of the reactor could commence. The timeline of each of the reactors is illustrated in Figure 2.

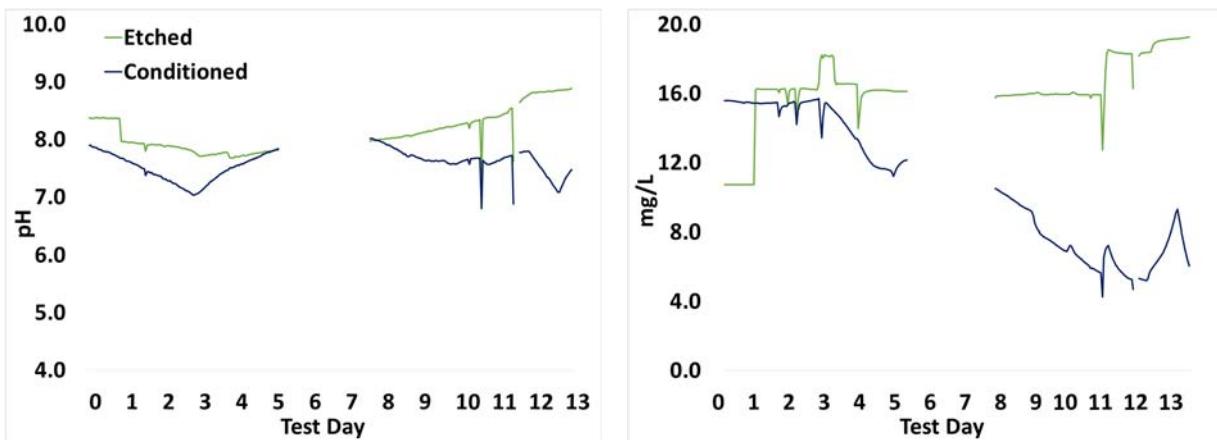


**Figure 3. An illustrated timeline of the inoculation of the etched (L) and conditioned (R) membranes.**

The etched membranes were successful in reducing the duration from inoculation to full wastewater operations by 32 days; however the system needed to be reinoculated for that to occur. After reinoculation, it took seventeen (17) days to be able to process urine, hygiene, laundry water, and humidity condensate.

The inoculation of the conditioned membranes performed significantly better than the initial AWP inoculation and the inoculation of the etched membranes. It took 13 days for the system to go from inoculation to complete wastewater operations, and did not require a second infusion of inoculum, which differed from the TTU protocol. This is the shortest period at which a reactor has been inoculated to date.

Why such a difference in between the etched and conditioned reactors? Figure 3, the inline pH and DO data, provide some information that may explain why.



**Figure 4. pH and DO concentrations through test day 13.**

The dissolved DO in the etched reactors never dropped below 14 mg/L, while the conditioned membranes, after a 3 day lag, dropped steadily, indicating bacterial activity in the reactor. After 21 days, the DO in the etched reactor was still above 14mg/L and was subsequently drained and re-inoculated. The air pressure was lowered mitigate the elevated DO. That allowed the consortium to adapt to the reactor, and the system completed the inoculation protocol. (Note: although the inline sensors lost communication with the log computer from test days 5 and 6, daily pH and DO data was still collected from the probes).

A potential cause of the elevated DO in the etched reactors may be due to chemical changes to the membrane after exposure to the etching solution. In addition to changing the surface to allow the microorganisms to readily attach, the solution may have also led to physical and/or chemical changes to the tubing to make more gas permeable. Baker and others have demonstrated that porosity of membranes can be increased through physical or chemical etching<sup>4</sup>. An increase in DO was not observed during KSC sub-scale testing; however, the large surface area of the MABR would magnify any small changes in permeability of the individual tubes.

Conversely, the conditioned membranes were able to decrease inoculation time without affecting gas permeability, and subsequently, DO concentration. When bacteria colonize a surface, they excrete materials to make it favorable for the cells to attach. Once the cells begin to attach, additional cells are introduced and/or produced, which subsequently produce additional extra cellular substances in order for the biofilm to mature. These substances can etch the surface of the membrane or make it more “sticky,” keeping bacteria adhered to the surface<sup>5</sup>. Although the initial biofilm was washed off, alteration of the surface remained, likely along with small numbers of tightly adhered cells on that adhesive surface, which allowed for rapid re-colonization. Analysis of both types of tubing is in work and will be presented in a subsequent paper.

#### IV. Loading Study

##### A. Testing and Results

Reducing the mass and footprint of a ECLSS system is also a potential barrier to implementation, so identifying the optimal size of a bioreactor to sufficiently process the wastewater is key. After almost two months of steady-state testing, the test team decided to challenge the loading of the bioreactor with the conditioned membranes to determine the upper capacity of a “new” reactor. The increase occurred in a stepwise manner, starting at a 1-person equivalent load and ending when either 1) the reactor was unable to oxidize the organic carbon or convert the ammonium in the wastewater, or 2) it was able to successfully process a 4-person equivalent wastewater load, whichever came first. Table 3, is an overview of the test points during this study.

**Table 3. Test points for challenge study**

Test Point	Dates	Volume Processed per Day (L)	Equivalent Person Wastewater Load
1	7/22-9/11	14.5	1
2	9/12-9/15	17.2	1.2
3	9/16-10/16	28.8	2
4	10/17-10/26	43.2	3

3a	10/27-11/13	28.8	2
----	-------------	------	---

Test point 1 was the baseline. It began at the end of inoculation once the reactor started processing a complete wastewater (urine, hygiene, laundry, etc.) and lasted for 52 days. Test point 2 was to be a two person equivalent load, but due to a software issue over a weekend, the load could only be increased to 17.2 liters per day or a 20% increase in loading. The remaining test points were either a “person-increase” or “person-decrease in loading.” Figure 4 summarized the TOC oxidation, ammonium removal, and grams ammonium removed per day.

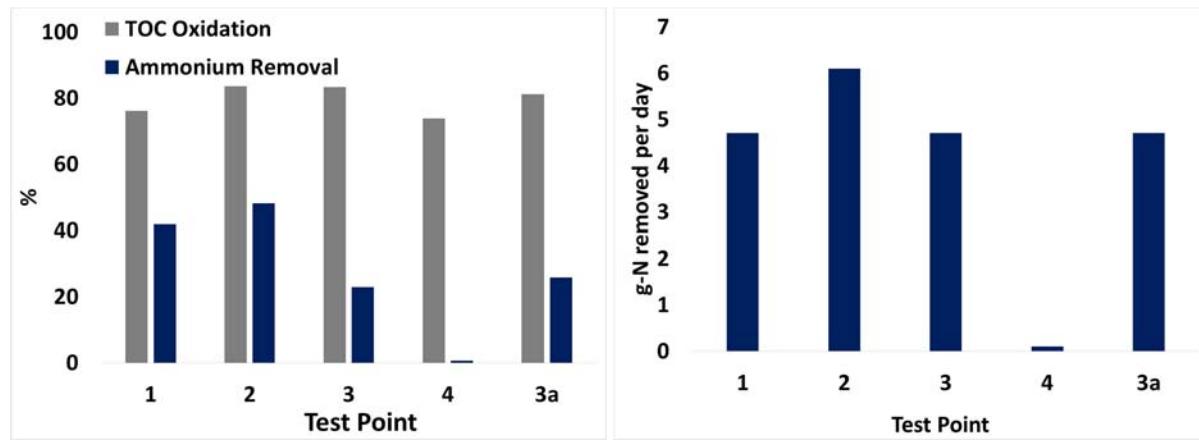


Figure 5. Summary of loading study results.

The reactor was able to consistently remove the majority of organic carbon from the influent, oxidizing between 74 and 84%; however ammonium removal dropped dramatically between test points 2-4, dropping from 48% ammonium removal to 1%. Normalizing for flow rate, the nitrogen removal remained above 4.5 grams nitrogen removed per day for test points 1-3, but dropped to nearly zero ammonium removal during test point 4. After nine 9 days at a three person equivalent wastewater load, the test team reduced the influent flow rate and repeated test point 3 (3a) until the end of testing. During the 17 day period, the reactor did rebound and performed similarly to test point 3.

Despite low nitrogen removal rates during testing, size optimization of the system is still possible. Since nitrification is the limiting factor in a SND process, nitrogen loading rates were used to size the reactor. As a part of the initial test design, the test team assumed an 80% conversion of NH<sub>4</sub>-N at an influent concentration of 550 mg/L and influent volume of 90 liters/day, a conservative volume assuming one large laundry load per day. At those parameters, 40m<sup>2</sup> of membrane surface area was needed to process the wastewater. The concentration of nitrogen for the loading study was 665 mg/L NH<sub>4</sub>-N, approximately 21% larger than the baseline, but the test volume (58L) was 36% smaller than the baseline. Assuming an 80% conversion of NH<sub>4</sub>-N, the surface area of the system drops 23% to 31m<sup>2</sup>; however decreasing the size is dependent on increasing the efficiency of ammonium removal.

The lower than expected removal rates is likely due to a low carbon to nitrogen ratio (C:N) in the influent, specifically, starving the nitrifying bacteria of the inorganic carbon needed for nitrification. Most studies have demonstrated a C:N between 3-5:1 as optimal for nitrification; however testing at JSC has demonstrated that much lower ratios were sufficient for high ammonium conversion. The influent processed in the integrated test in 2000-2001 had a C:N ratio of approximately 0.8:1 and averaged 75% removal in NH<sub>4</sub>-N. The influent during the loading study had a C:N of roughly 0.5:1. It is thought that the infusion of additional carbon producers (e.g. solid waste leachate) will provide the necessary carbon to optimize nitrification and provide an effluent with minimal ammonia-nitrogen and organic carbon. Currently the test team is investigating the influence of additional carbon producers on reactor performance and effluent quality and will present this data in a subsequent paper.

## V. Conclusion

The ability to rapidly inoculate a reactor and transition to nominal operations, as well as the ability to minimize the size of the reactor are necessary for a biologically-based water recovery system to be considered for a given ECLSS mission architecture. Results from testing demonstrate that a membrane bioreactor can go from inoculation to operation in under two weeks, using the TTU inoculation protocol and conditioned membranes. In addition, the system was able to successfully process a partial wastewater influent (i.e. no surfactants) as a part of the inoculation process, and therefore is able to produce a product within days. Testing also demonstrated that the system size can be reduced on the order of 20%, given increases in ammonium removal. Analysis is in work to determine whether chemical or mechanical changes in the membranes as a result of the etching led to an increase in gas permeability and additional testing will take place to confirm evaluate the role of additional carbon on performance, and subsequently, sizing of a system.

### Acknowledgments

The authors of this paper would like to thank Tyler Garabaldien and Giraldo Alvarez for assisting in the day-to-day activities of the AWP test, Chris Carrier and Stacey Moller for their assistance in performing the analyses of the many samples during testing, and the many wastewater donors in the Life Support and Habitability Branch and the Crew and Thermal Systems Division.

### References

<sup>1</sup>Pickering KD, Mitchell JL, Adam NM, Barta D, Meyer CE, Pensinger S, Vega LM, Callahan MR, Flynn M, Wheeler R, Birmele M, Lunn G. Alternative Water Processor Test Development. 2013. 43<sup>rd</sup> International Conference on Environmental Systems

<sup>2</sup>Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG. "Inhibition of Nitrification by Ammonia and Nitrous Acid," *Water Pollution Control Federation*, Vol. 48, No. 5, May, 1976, pp. 835-852

<sup>3</sup>Sevanthi R, Christenson D, Cummings E, Nguyen K, Morse A, Jackson WA. Performance of a Full Scale MABR (CoMANDR 2.0) for Pre-treatment of a Habitation Waste Stream Prior to Desalination. 2014. 44th International Conference on Environmental Systems.

<sup>4</sup>Wheeler R, Lunn G, Hummerick M, Johnsey M, McCoy L, Koss L, Dominguez J, Richards R, Ruby AM, Larson B, Monje O, Mellott K. "Biological Pretreatment for Stored Wastewater. 2013 End of Year Annual Report." ESC-111-BWP-008, 2013.

<sup>5</sup>Costerton JW, Lewandowski Z, Debeer D, Caldwell D, Korber D, James G. "Biofilms, the Customized Microniche," *Journal of Bacteriology*, Vol. 176, Issue 8, April 1, 1994, pp. 2137-2142.

<sup>6</sup>Baker, R., "Membrane Technology," *Encyclopedia of Polymer Science and Technology*, John Wiley and Sons, Inc, New York, NY, 2002, pp. 184-249.

<sup>7</sup>Campbell ML, Finger, BL, Verostko, CE, Wines, KR, Pariani, GM, Pickering, KD. Integrated Water Recovery System Test. 2003. 33<sup>rd</sup> International Conference on Environmental Systems